

### **Abstract**

The present invention provides methods for detecting the presence and enzymatic activity of reverse transcriptase (RT) in a sample. The methods generally involve conducting a reverse transcriptase PCR assay in the presence of labeled deoxynucleotides. The deoxynucleotides are incorporated into a molecular structure or complex containing the RNA template and the extending cDNA primer. In one embodiment the deoxynucleotides are labeled with a detectable moiety. A capture moiety can also be included to immobilize the complex on a surface after completion of the reaction and facilitates detection of the molecular structure, and therefore the presence of reverse transcriptase. In one embodiment the detectable moiety is a chemiluminescent moiety, such as an acridinium dye, and the assay is determined by stimulating chemiluminescence from the detectable moiety and detecting light emitted. In various embodiments the assays are also useful for determining the sub-type of reverse transcriptase present in a sample, or for screening for anti-retroviral lead compounds. Also disclosed are kits for conducting the assay.